University of California at Berkeley Department of Nutritional Sciences

Semi-Annual Progress Report

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Title: Nutritional Requirements and Breeding Behavior of Perognathus

1. Nutritional Requirements:

These past months we have been concerned with the development of a semisynthetic diet which will maintain weight, good health and normal activity patterns in two subspecies of Perognathus (Longimembris and Penicillatus).

a) As a first step the composition of the diet on which the animals seem to thrive indefinitely was analyzed. The gross composition of this diet composed of 1 part sunflower seed and 1 part millet is shown in Table 1. The stock colony is maintained on a mixture of sunflower seed, canary seed, oats and millet. The gross composition of this diet is very similar to that given in Table 1 because the animals hull each seed carefully before consumption. Food intake was measured. The average food intake per day of a 20-g P. penicillatus was 1.5 g, equal to 0.075 g/g of body weight. The calculated intake of macronutrients is given in Table 2.

We have also kept animals on diets of single seeds for long periods of time. These seeds included sunflower, safflower, millet, rice, wheat and oats. All groups, each composed of 2-4 P. longimembris, have done well for three months although the composition of these seeds varies widely (the oil seeds average 45% fat and 20% protein; the cereal seeds average 3-7% fat and 10-12% protein). While this does not prove that all of them are ideal diets, it does show that each of them will support life for long periods of time.

b) We have tried a commercial mouse pellet based on natural ingredients (Table 3). The animals seemed to do well. After approximately 16 weeks they suddenly lost weight and most of them died within a few days. A slowly developing deficiency or cumulative effects of an imbalance of nutrients must be assumed to be the cause. The experiment did show however that a pelleted diet is acceptable to these animals. Another commercial mouse pellet of different composition is being presently tried.

c) As a next step in the development of a defined diet, a pellet composed of 50% sunflower meal and 50% millet flour was tried (composition is the same as the mixture of the seeds (Table 1)). This diet (S-M-D) is very well accepted and the animals (6 P. longimembris, 1 P. Bailey) are thriving on it since 5 months.

d) We then tried a semisynthetic diet (Table 4). The animals lost weight and some died within 10-15 days, although they consumed the diet as shown by full intestinal tracts found at autopsy. In order to determine whether any single component of this diet was responsible for the inadequacy, the following modifications were tried, all of which also failed: low fiber content (2%), soy protein instead of casein, sunflower oil instead of cottonseed oil,

dextrose or arrowroot starch instead of corn starch, yeast instead of B vitamin mix, a commercial safflower protein meal* instead of casein and fiber, whole dry milk replacing the casein and some of the cottonseed oil, and complete elimination of starch, being replaced with soy protein or casein. Supplementation of the diet with fresh carrots did improve the performance greatly; this point is under further investigation at this time.

The addition of whole sunflower meal was attempted. At 10% and 20% the diet still proved inadequate; at 30% the improvement was noticeable; at 40% the diet was successful.

e) The success of the sunflower meal and the failure of the synthetic diet can be due to either a harmful or toxic effect of some component(s) in the synthetic diet or to the presence of an essential nutrient or the proper balance of nutrients in the sunflower meal. To study this problem we have:
1) added the individual components of the synthetic diet to sunflower meal pellets, and
2) added individual fractions of sunflower meal to the synthetic diet.

The addition of either 25% casein, 40% corn starch, the vitamin mix, the mineral mix or the non-nutritive cellulose fiber to sunflower meal appeared not to decrease its adequacy. The experiment concerning mineral mix has not been continued long enough as yet to be conclusive (7 weeks).

Sunflower meal was fractionated into the oil-soluble, the nonoil-soluble and ash components. A purified sunflower protein (globulin) has also been prepared. The oil-soluble portion when used to replace the cottonseed oil in the synthetic diet did not improve it. We are now in the process to test the other components (nonoil-soluble fraction, purified protein and minerals).

It appears likely as of now that the problem is centered around the water and/or mineral or protein nutrition. Another factor which must not be overlooked is the possibility that these animals depend on a highly adapted intestinal flora which is maintained by certain components of a natural diet.

2. Manipulation of the Climatic Factors of the Environment:

We have received, installed and checked out the performance of a "climatron" (Sherer-Gillette). Our plans are to subject groups of mice to "seasons" or seasonal changes as similar as possible to that which they experience in the field, hoping that this will induce breeding. The major difficulty of this approach is the scarcity of information as to the conditions to which nocturnal, burrowing desert animals are actually exposed and to the minimum of time necessary in each "season" to elicit the normal physiological response. Our experimental designs are therefore based on more or less well-informed guesses.

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* This safflower protein meal is very high in coarse fiber (15%) and is a by-product of safflower oil extraction after partial decortication of the seeds. The failure of the diet containing this product is probably due to the high content of coarse fiber.

3. Physiological Characteristics:

a) Unexpected periodic weight fluctuations have been observed in all four subspecies under observation, when fed the mixed seed diet (p. 1). These conconsisted of periods of 50-60 days during which the body weight was 15-25% above or below average. The majority of the periods of high body weight occurred in May-June and August-September, while the majority of the periods of low body weight occurred in June-July and September-October. Individuals of P. Baileyi and P. penicillatus showed this periodicity more consistently than did P. intermedius and P. longimembris. The cause of these fluctuations is obscure. We are now testing the possibility that the animals select different seeds out of the mixture during different periods, by feeding one group the mixture of sunflower seed and millet (Table 1) and another group the pelleted SMD diet of the same composition.

b) In order to obtain baseline values for future work we have determined organ weights, carcass composition and certain blood characteristics of a group of mice. The results are shown in Tables 5,6. We are now doing a thorough histological study of the organs of these mice.

Plans for the Immediate Future--In addition to the studies outlined in 1-3 above we are beginning hormone treatments of the mice in an attempt to induce estrus and mating. We are planning to treat both males and females and to administer the hormones both orally and by injection.

Summary:

1. In order to study the nutritional requirements we have developed a pelleted diet from sunflower meal and millet which is adequate to maintain weight and general health in P. penicillatus and P. longimembris. We are now studying the problems involved in developing a semisynthetic diet.

2. In order to study the breeding behavior we have obtained a "climatron." We are planning to use it to subject mice to the seasonal changes which they experience in the field in the hope to elicit the physiological responses necessary for breeding.

3. In order to obtain baseline values for future studies we have determined organ weights, carcass composition and certain blood values of a group of P. penicillatus.

Table 1. Composition of Seed Mix (½ sunflower, ½ millet - hulled)

Protein	Fat	Carbohydrate	Fiber	Total Ash	Water-sol. Ash	Water
%	%	%	%	%	%	%
18	25	50	3-4	3.7	1.0	9.0

Table 2. Calculated Daily Intake of Macronutrients[1]

Protein	Fat	Carbohydrate	Minerals	Calories
mg	mg	mg	mg	Kcal
270	380	900	60	7.7

[1] *P. penicillatus*, body wt. 20 g.

Table 3. Composition of Purina Mouse Breeder Chow

Protein	Fat	Fiber	Ash	Water
%	%	%	%	%
18	12	2	5	9

Ingredients:

Nonfat dry milk, ground wheat, yeast, vegetable oil, animal fat preserved with BHT, vitamin A supplement, ferric-ammonium citrate.

Table 4. Composition of a Semi-purified Diet (modified Bell's diet)[1]

Casein	22.3	g/100 g diet
Corn starch	44.3	"
Sucrose	5.2	"
Cottonseed oil	9.0	"
Fiber	11.2	"
Mineral mix		
$CaHPO_4$	1.2	"
$CaCO_3$	2.5	"
NaCl	1.3	"
KCl	0.4	"
$MgSO_4.7H_2O$	0.1	"
Fe citrate	0.1	"
Traces of cobalt, copper, manganese and iodine		
Vitamins		
Choline	135	mg/100 g diet
Riboflavin	0.4	"
Thiamine	0.3	"
Niacin	0.3	"
Pyridoxine	0.1	"
Folic acid	2.5	"
Biotin	0.01	"
Inositol	0.1	"
PABA	1.2	"
Menadione	10.0	"
Tocopherol	6.0	"
B_{12}	0.5 ug	"
A	50 IU	"
D	20 IU	"

[1]NRC Publ. 990, p. 46: Nutrient Requirements of Laboratory Animals.

Table 5. Carcass Composition

Carcass lipids: 15.0% of fresh wt. (2 P. pen.)

Carcass ash: 3.0% of fresh wt. (2 P. pen.)

Table 6. Blood Chemistry

	Blood sugar	Serum protein	RBC	WBC	Differential count %			
	mg %	gm %	x10^6/mm^3	per mm^3	Mono-cytes	Lympho-cytes	Segm.	Baso-phil
Av.[1]	120	3.96[2]	8.8	2600	2	85	13	0
Range	93-147		8.1-9.5	2100-2900	0-7	75-95	5-20	0

[1] 3 P. pen., 1 P. inter

[2] 1 pooled sample